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Toxicity assays of ladybirds using natural predators

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Abstract. Nestling blue tits *Parus caeruleus* L. were given diets containing homogenized ladybirds, to assess the effects of their chemical defences. The 2spot, 10spot and water ladybirds produced no apparent toxic effects when small numbers were given at regular intervals.

The pine and kidney-spot ladybirds slightly inhibit growth and may be toxic to very young nestlings. The Water ladybird is extremely distasteful to this predator, despite its lack of toxicity. The results are discussed with reference to the proposed Müllerian and Batesian mimetic relationships between the ladybird species.

Key words. chemical defence – toxicity – mimicry – Coccinellidae – *Adalia bipunctata* – *Adalia decempunctata* – *Exochomus quadripustulatus* – *Chilocorus renipustulatus* – *Anisosticta 19-punctata* – *Parus caeruleus*

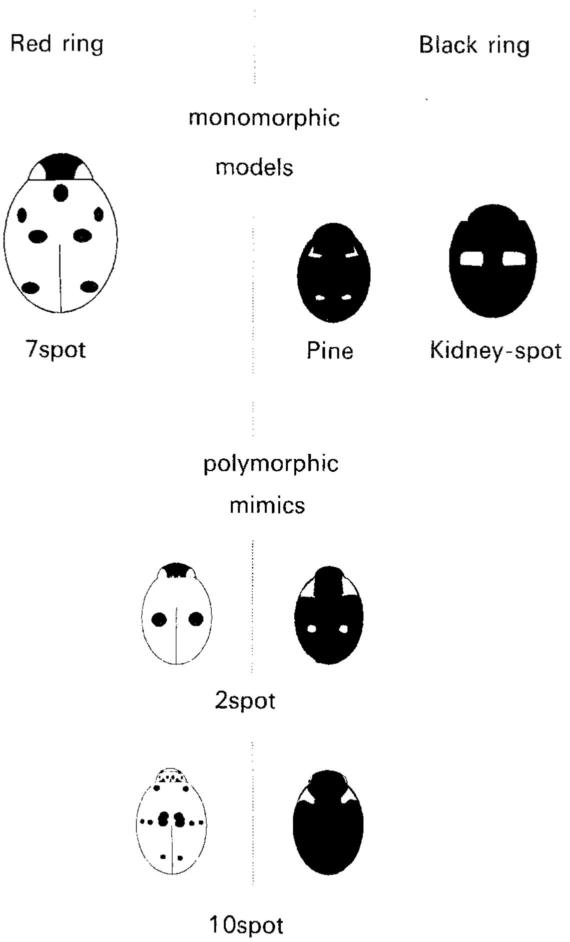

Fig. 1 Schematic representation of the proposed mimetic relations between the most widely abundant European ladybird species. (After Brakefield 1985)

Introduction

It has been suggested that monomorphic European ladybird species, such as the 7spot ladybird *Coccinella septempunctata* and the pine ladybird *Exochomus quadripustulatus*, are aposematic members of Müllerian mimicry rings (Brakefield 1985; Marples 1990). These species are the commonest members of their respective rings, one based on a colour pattern which is red with black spots (7spots), the other being black with red spots (pine ladybird). A second possible member of the black ring is the monomorphic kidney-spot ladybird *Chilocorus renipustulatus* (see Fig. 1). Certain polymorphic species of ladybirds have colour morphs very similar to these Müllerian mimicry rings. These may be Batesian mimics of the above monomorphic species Brakefield (1985). Two very common polymorphic ladybird species are the 2spot *Adalia bipunctata* and the 10spot *Adalia decempunctata*, each having a red and at least one black morph. The water ladybird *Anisosticta 19-punctata* is temporally polymorphic, the background to its black spots changing from dull buff in winter, to bright red during the spring. In winter it is cryptic on dead reed stems, while in spring the green shoots and the ladybird's greater activity, make camouflage much more difficult. If these polymorphic species are mimics and the monomorphic species models, a difference in toxicity between the two would be expected; the Batesian mimics being non-toxic, although possibly distasteful, while their models would be toxic (Turner 1987; Huheey 1988; Pough 1988 for recent reviews). The models in mimicry rings need to contain toxic chemicals, since it has been shown that predators may avoid unpalatable species initially, but eventually learn to accept prey lacking an actual toxic effect (Muhlmann 1934; Huheey 1984; Marples 1990).

All these ladybird species contain large quantities of alkaloids (Pasteels *et al.* 1973; Holloway *et al.* 1991; de

Jong *et al.* 1991) although not the same alkaloid in each case. Both the *Adalia* species contain adaline. The pine ladybird contains exochomine, the water ladybird hippodamine, and the 7spot ladybird contains coccinelline and its precursor precoccinelline (Pasteels *et al.* 1973). The kidney-spot ladybird contains an alkaloid (Marples 1990) although (to my knowledge) it has not yet been identified. These chemicals taste bitter to humans and are often assumed to be defensive (Tursch *et al.* 1976; Moore & Brown 1978; Boppré 1986; Holloway *et al.* 1991; de Jong *et al.* 1991).

Marples *et al.* (1989) demonstrated that the 7spot ladybird is extremely toxic to blue tit nestlings *Parus caeruleus* L. The red (*typica*) form of the 2spot ladybird, on the other hand, is totally non-toxic to this predator (Marples *et al.* 1989). This supports the hypothesis that these species are model and mimic respectively (Brakefield 1985). The present study was carried out to determine whether a similar toxicity relationship exists between the proposed models and mimics within the black mimicry ring. The model species, the pine and kidney-spot ladybirds and the polymorphic Batesian mimics 2spot and 10spot were used. The results of a pilot study using the water ladybird are also presented.

Materials and methods

Treatments

The collection, raising and return of the blue tit *Parus caeruleus* L. nestlings to the wild followed the procedures outlined in Marples *et al.* (1989). Chicks were randomly assigned a treatment within their sibling group, dictating which ladybird species was added to their diet. They were fed two types of pellets, treatment pellets and standard food pellets, made in the manner described by Marples *et al.* (1989). Treatment pellets contained standard food plus one of the ladybird species, or mealworm *Tenebrio molitor* for the 'control' treatment.

Table 1 Sibling groups of nestling blue tits and the species added to the diet of each bird: mealworm (control); 2spot ladybirds of each morph (*typica* and melanic); 10spot ladybird (10spot); pine ladybird (pine); kidney-spot ladybirds (kidney); and water ladybirds of each morph (water (red) and water (buff)). Nest 7 contained 5 birds taken from two families

Year	Nest	Sibling Birds			
		1	2	3	4
1988	1	control	*typica*	10spot	pine
	2	control	*typica*	10spot	pine
	3	control	*typica*	10spot	pine
	4	control	*typica*	melanic	pine
	5	control	*typica*	melanic	pine
	6	control	*typica*	melanic	pine
	7	*typica*	water (red)	water (buff)	
		typica	melanic		
1989	8	control	kidney	pine	
	9	control	kidney	pine	
	10	control	kidney	pine	pine

The treatments offered, and the arrangement of sibling groups in both years is shown in Table 1. The numbers of birds tested with diets containing kidney-spot, 10spot, melanic 2spot and water ladybirds were all restricted by the numbers of beetles available. The red ('*typica*' treatment) and the black ('melanic' treatment) colour morphs of the 2spot ladybird were tested separately. As numbers of 10spot ladybirds were limited, all the colour morphs were mixed together. One nestling was given a diet containing the red morph of the water ladybird, and a second was given the buff morph. This ladybird is very small, so three per pellet were required to equal the mass of one 2spot, but as this ladybird is 'locally common' (Majerus & Kearns 1989) reed-bed nesting birds might frequently encounter such numbers.

The birds on the *typica* and control treatments acted as a general control to indicate comparability between Marples *et al.* (1989) and this study, as well as an estimate for the maximum growth rate expected.

Experimental procedure

The experimental procedure was largely that described by Marples *et al.* (1989), but a few alterations were made. At 4-hourly intervals each nestling was weighed, and the leg and wing lengths were measured. The wings were measured from the foremost extremity of the carpus (x) to the tip of the wing, or the longest primary feather (y) once these had started to grow (Fig. 2A). The legs were measured when fully extended, from the angle of the intertarsal joint (x) to the tip of the longest toe, excluding the claw (y) (Fig. 2B).

For four days, each hour of the 16 h day the birds were given a double feeding round, consisting of a treatment pellet and two rounds of standard food pellets. The treatment pellet was placed on the tongue using forceps. Refusal to eat the treatment voluntarily was recorded, but the pellet was then pushed to the back of the throat to ensure that it was eaten. After their treatment pellet, the birds were offered the first feeding round of *ad libidum* standard food pellets, and water, followed by a 10 min rest, then a second round the same as the first. The rank heights of the birds' heads when they begged were scored before the treatment pellet was given, and as the second feeding round began. Those which failed to beg scored zero, and the highest head scored four. Where there were not four birds in the nest

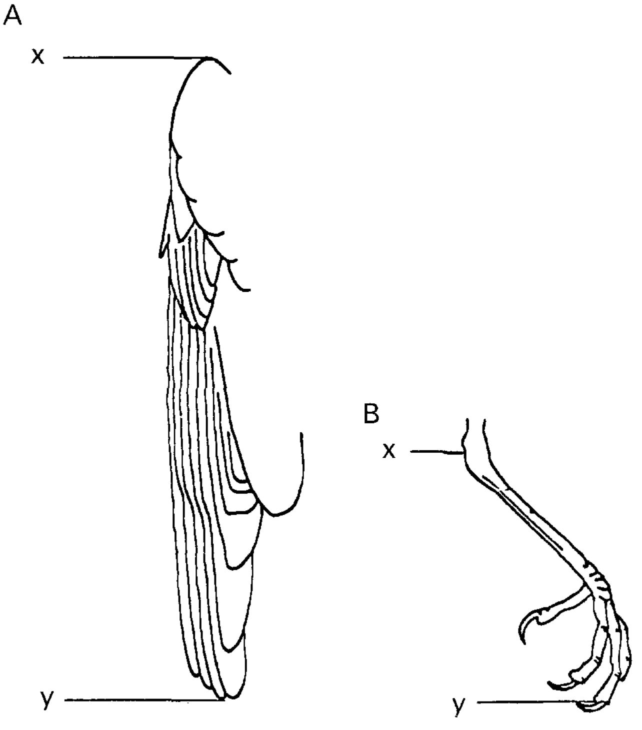

Fig. 2 **A** Passerine wing and **B** passerine leg showing the points between which measurements were taken for wing and leg growth

the begging scores were given as fractions such that the range available was zero to four. The number of food pellets eaten by each bird and the number of faecal sacs they produced were recorded at each feed. The weight of faecal sacs produced from each diet was also recorded.

Results

The mean initial weights of the nestlings in each treatment were not significantly different ($F_{3,24} = 0.49$) so no initial bias was present towards one treatment or another. As the pine ladybird came from various sites, samples from each site (Holland, Cardiff and Cambridge hibernating populations), and samples of pellets used in a pilot experiment by Marples *et al.* (1989) were tested for the presence of alkaloid using Dragendorf reagent. Alkaloid was found in all the samples, with no consistent difference in staining intensity between the samples so the samples were pooled. There was also no difference in alkaloid content of the beetles collected in either year of the experiment.

Survival

There was no effect of treatment on survival as all the birds remained healthy throughout the experiment. The two-round feeding system increased the total number of pellets eaten by each bird at a feed compared to the single round used by Marples *et al.* (1989), keeping their weights very close to that of their wild siblings.

Weights

While an analysis of variance shows no significant difference in either the total or daily mean weight gains by birds on the melanic, *typica*, 10spot, water ladybird or control treatments, birds on both the two proposed black ring models gained weight less rapidly than their control and *typica* fed siblings (Fig. 3A). The difference was significant after one day on the treatments, ($F_{3,24} = 8.62$, $P < 0.01$).

There was no significant nest or year effect in the data pooled between the two years.

Leg and wing growth

As measurements of these parameters varied by up to a millimetre in either direction in consecutive readings, the average for each bird over a day was calculated. There was no significant difference between any of the treatments, but a highly significant nest effect for both parameters (Leg length $F_{7,21} = 4.94$, $P < 0.01$; Wing length $F_{7,21} = 123.97$, $P < 0.001$).

The birds on the pine ladybird and kidney-spot treatments continued to grow wings at a fast rate over the whole experiment while the other treatments showed a slower rate in the third and final days (Fig. 3B). The control fed birds were also the first to show a reduction in the rate of leg growth, slowing on the third day.

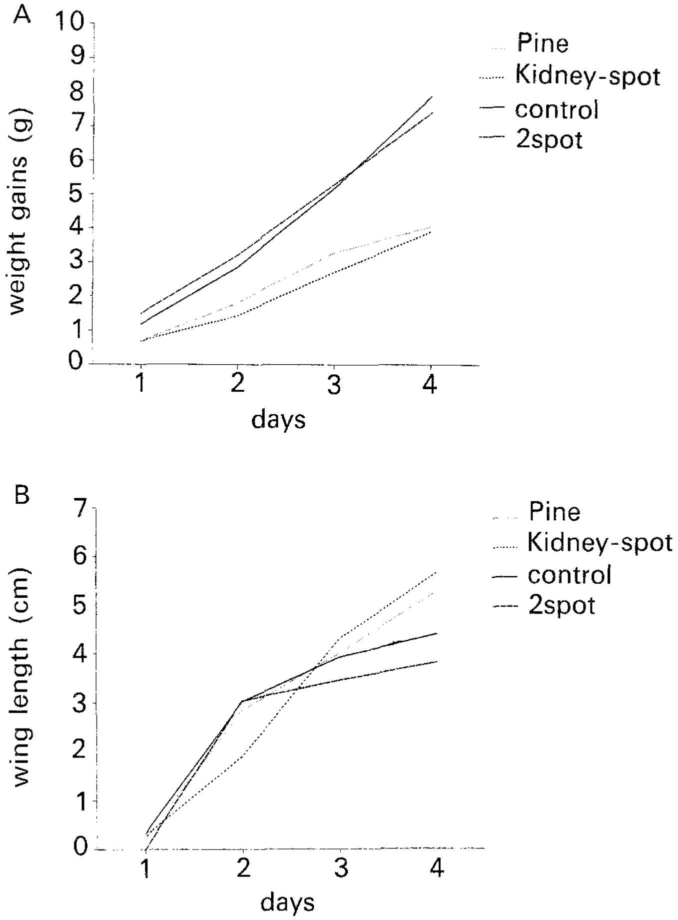

Fig. 3 Effect of diets containing the black ring species and their silbing controls on growth rate parameters in nestling blue tits. **A** daily weight gains; **B** daily increases in wing length

Food intake

There was no overall significant difference among treatments in the number of food pellets accepted by the end of the experiment, although, on the second day the *typica* treatment birds ate significantly more than their siblings ($F_{3,24} = 7.67$, $P < 0.01$). All the ladybird treatments were higher than control on that day.

The percentage of treatment pellets which were rejected are plotted for all the birds (Fig. 4). Far more rejection of the water ladybird pellets was found than of other treatments ($\chi^2_6 = 297.93$, $P < 0.01$). This response was restricted to the treatment pellets, and was so marked that it leads one to class the water ladybird as very unpalatable despite being based on observations from only two birds. The water ladybird pellets were rejected far more often even than 7spot pellets (Marples *et al.* 1989) ($\chi^2_1 = 15.5$, $P < 0.01$), while rejection rates of *typica* and control pellets in the two experiments were comparable. It is also clear that the pine ladybird pellets were regarded by the nestlings as distasteful and were rejected more often than the other treatments ($\chi^2_3 = 10.9$, $P < 0.05$). This was strongly influenced by a single bird (12 of the 26 rejections), nonetheless rejection was widespread with only one bird out of the ten failing to reject at least one pine ladybird pellet.

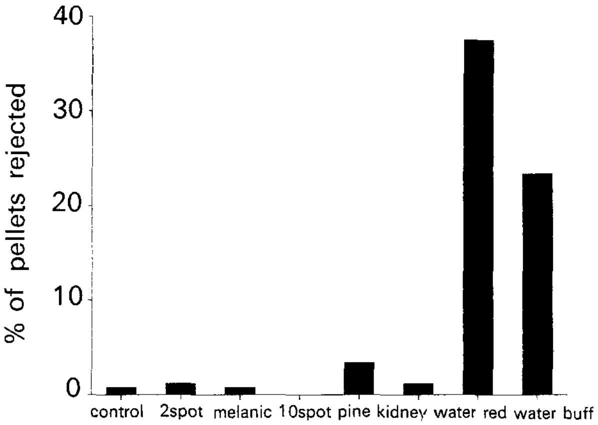

Fig. 4 Percentage of the food pellets containing various species of ladybird which were initially rejected by nestling blue tits

Defecation

Although on the first day there was a difference between the treatments in the number of faecal sacs produced ($F_{3,24} = 3.28$, $P < 0.05$), this effect had disappeared by the second day. The weights of the sacs (Fig. 5) show that only the pine and kidney-spot treatments produced sacs as light as the control diet, the others all being considerably (but non-significantly) heavier.

Begging scores

There were no significant differences between treatments in the amount birds begged on either feeding round, even on the second day when the birds on *typica* treatments ate more pellets. The birds on black ring diets begged consistently less than the other treatments throughout. Those on the pine ladybird diet did not reduce their begging score on the final day like the birds on other treatments, but increased their begging rate in the first feeding round and maintained their second round begging at a steady level.

The birds fed the water ladybird diet ate more pellets and begged less than the other birds. However, as there were only two birds on this treatment, and they were siblings, this could be a reflection of healthier birds, independent of the treatment.

Marples *et al.* (1989) found a far higher begging rate among *typica* fed birds, but no such effect was seen in this data set.

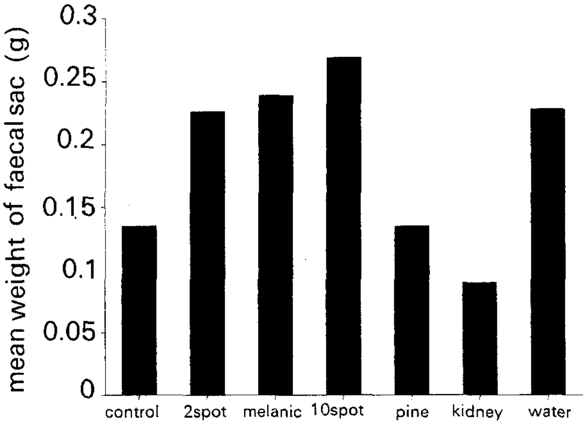

Fig. 5 Mean weights of faecal sacs produced by nestling blue tits when their diets were supplimented by various species of ladybirds

Discussion

These experiments tested the toxicity of the proposed black ring models, the pine and kidney-spot ladybirds, and the most common Batesian mimics, the 10spot and 2spot, together with temporally polymorphic species, the water ladybird. The results provide evidence of a greater toxicity in the black ring model species than in the putative Batesian mimics, but that none of these species is as toxic as 7spots, the most common model in the red mimicry complex. This is consistent with the view (Brakefield 1985) that the red mimicry ring derive better protection from mimicry than the black ring mimics, the latter being affected more by non-mimetic advantages of melanism.

To function as a fully effective model, an insect needs to be detrimental to the predator, since degree of protection is influenced greatly by the model's noxiousness (*e.g.* Alcock 1970; Goodale & Sneddon 1977). Abundance of the model also influences the effectiveness of the pattern (see for example Turner 1987; Huheey 1984) so the rarer black ring models might constitute a less effective model than the 7spot. There is also less spatial overlap between the mimics and the black models due to different habitat preferences in some populations (Majerus & Kearns 1989). On the other hand, their more similar size and pattern to the mimics, and the intrinsically more aversive nature of black (Schmidt 1960; Schuler & Roper 1992) could increase the effectiveness of the black ring models.

Despite these advantages, both pine and kidney-spot ladybirds seem less effective models for their 2spot and 10spot mimics than the 7spot. The universal survival of the nestlings in itself indicates that neither the pine ladybird nor the kidney-spot are as well protected chemically as the 7spot (Marples *et al.* 1989), at least against blue tits. A pilot study using two birds (Marples *et al.* 1989) showed a very toxic effect of pine ladybirds, both birds becoming too ill after two days for the treatment to continue. These birds were about half a gram smaller than most used in these experiments, which may have made them more susceptible. In the present study, the birds fed the pine ladybird diet ate no fewer pellets and produced less faeces, so their slower growth rates indicate that they were using the energy less efficiently for growth. Similarly, in Marples *et al.* (1989) the birds on the 7spot low dosage treatment showed a lower weight gain despite eating similar amounts and defecating the same amount as control birds. The birds on black ring diets begged consistently (but not statistically significantly) less than the other treatments throughout, suggesting that they were less healthy.

The chemical defences in the pine and kidney-spot ladybirds may retard development. Perrins (1979) cites data (O'Connor 1973) showing that the wing growth rate of blue tits is at a maximum between six and ten days, then reduces once the wings are fully grown. The graphs for birds on all except the black ring diets indicate such a reduction (Fig. 3B). All the birds except those on the pine ladybird diet also reduced their begging score on the final day.

Thus the chemical protection in the black ring models clearly deleteriously affects nestling development, and presumably fitness since the most important indicator of subsequent survival for titmice is fledgling weight (Perrins 1979). The toxin may also have some detrimental effects on older birds, if only due to the energy wasted in denaturing it, so it is unlikely that the adults would favour black ladybirds as a food source, even outside the breeding season. The apparent distastefulness of the pine ladybird would further add to their protection. Despite their low toxicity, therefore, these species cannot be said to be unprotected and may act as Müllerian mimics. However, as a primary model leading to the evolution and maintenance of the melanic morphs of the 2spot and 10spot ladybirds they seem insufficiently protected.

Other potential models exist in some habitats, such as the common and very well protected burnet moths *Zygaena* sp. (Sbordoni *et al.* 1979; Rothschild *et al.* 1984) and cinnabar moths *Callimorpha jacobaeae* (Marsh & Rothschild 1974). There is good evidence that birds may generalise from very different models to their mimics (Windecker 1939), and as birds travel over a number of habitats during their life, a very nasty lesson learned in one may well be applied in another.

While the maintenance of melanic morphs through mimetic associations is a possibility, many other factors have been proposed to contribute to the maintenance of the melanic morph of the 2spot. These include more successful breeding (Timofeeff-Ressovsky & Svirezhev 1966; Lusis 1961; Brakefield 1984a,b; O'Donald & Majerus 1984), advantages associated with thermal and industrial melanism (Lusis 1961; Creed 1966, 1971a; Lees *et al.* 1973; Brakefield & Willmer 1983; Brakefield & Lees 1987), greater stress tolerance (Creed 1971b; Marriner 1926; Merritt-Hawkes 1927), and superiority in maritime areas (Lusis 1961; Bengston & Hagen 1977; for discussion see Marples 1990).

It is interesting to note the high degree of distastefulness present in the water ladybird, despite their lack of toxicity. Birds eventually accept nasty tastes if they do not lead to subsequent illness (Muhlmann 1934; Huheey 1984; Marples 1990), so these ladybirds would only be protected while the birds were learning to accept the taste. However, if the taste mimics that of the other more toxic ladybirds, then chemical mimicry could be invoked (Brower 1984). The bright red morph of the water ladybird is only present during the summer when other food is plentiful, and this also restricts the time available for the birds to learn acceptance of the taste. It is possible that under such circumstances an obnoxious taste is a sufficient deterrent for a toxin to be unnecessary.

In addition to the nestling toxicity tests, an attempt was made to develop a bioassay avoiding the use of live animals, by incubating slices of rat liver in a medium containing ladybird extracts. This would have made comparisons within and between individuals easier and quicker, while avoiding any suffering and disturbance that may have been caused by the nestling toxicity tests (although, of course, rats still had to be sacrificed to provide the livers). Unfortunately these assays showed limited success as some rats appeared to be very sensitive to the ethanol in which the extracts were dissolved, giving very variable results. No medium in which the extracts would dissolve was found which did not affect the livers. Thus, although the use of such a bioassay was seen as advantageous in many ways, it was not as reliable as the assay using wild nestlings.

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